Neugenesis
A Biotechnology Company

COMBINATORIAL BIOLOGY TECHNOLOGY

Neugenesis' combinatorial biology technology (the CombiKARYON™ system) mimics the immune system's ability to generate diversity in antibodies, and expands the application to all heteromeric proteins. The company uses this technology to discover and improve complex proteins. It is an efficient and cost-effective method to improve a protein's stability, affinity, receptor binding capacity, and therapeutic efficacy, thereby enhancing the value of the protein, and decreasing the risk of clinical failure.

Using Combinatorial Biology to Generate Diversity

An outgrowth of Neugenesis' protein production systems, CombiKARYON™ uses the unique features of the filamentous fungus, *Neurospora crassa*, to create combinatorial panels of heavy and light chains of a heteromeric protein and to build libraries of diverse, new, fully assembled proteins. Variants of each subunit gene are generated within the host by Neugenesis' proprietary technology. Strains carrying these new gene sequences are fused to one another in all possible combinations to produce libraries in the following manner.

VARIANT	Light chain 1	Light chain 2	Light chain 3	Light chain 4
Heavy chain 1	L1H1	L2H1	L3H1	L4H1
Heavy chain 2	L1H2	L2H2	L3H2	L4H2
Heavy chain 3	L1H3	L2H3	L3H3	L4H3
Heavy chain 4	L1H4	L2H4	L3H4	L4H4

In this illustration, 16 unique monoclonal antibody combinations are produced from 4 light and 4 heavy chain subunit variants. In a standard microtiter plate configuration, 96 unique combinations would be produced when 12 variants of one subunit are arrayed against 8 variants of the second subunit. With CombiKARYON™, this would be done with 20 total transformations. Traditional protein engineering techniques would require 96 transformations after a complicated reassembly process of the subunit genes. These burdensome steps are eliminated using Neugenesis' combinatorial biology approach. The advantages become more apparent in larger libraries. For example, a 100x100

matrix to create 10,000 combinations would require 200 transformations in the CombiKARYON™ system, and 10,000 transformations using traditional techniques. This technology can also be used to create combinations of more than two subunits, to geometrically increase the diversity. The last step is to screen the combinatorial libraries for new proteins with the desired characteristics.

Applying Combinatorial Biology to Drug Discovery and Improvement

CombiKARYON™ is an expedient approach for companies involved in developing difficult and complex protein therapeutics. The applications of the technology are numerous. For example, Neugenesis' technology may be applied to protein hits to improve the characteristics such as binding capacity or stability. By designing and creating small changes in the original molecule, Neugenesis is able to fine-tune the protein without dramatically changing the protein's core structure, which has already been selected for through years of evolution. This technology can also be applied to protein drug candidates already in pre-clinical and clinical trials. The failure rate of drug candidates in the development process is estimated to be at least 60-80%. Neugenesis' combinatorial biology system may help by providing a means to more efficiently fine-tune these candidates into better, therapeutically useful molecules.

Other potential applications of CombiKARYON™ include drug combination research and hybridization. In drug combination research, combinatorial biology enables rapid and inexpensive creation of any number of combinations of synergistic proteins, which can then be screened for the most effective combination. In hybridization, combinatorial biology can be used to develop hybrid molecules with both binding and effector moieties, improving the specificity of therapeutic agents.

In addition, cultures of desirable molecules identified through this technology can be easily expanded to produce large-scale quantities of the new heteromeric protein for further evaluation, since the protein is already in a *Neurospora* production strain.

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Glossary of Terms Used in Medicinal Chemistry
(IUPAC Recommendations 1998)

A to H

Contents

Active transport*

Active transport is the carriage of a solute across a biological membrane from low to high concentration that requires the expenditure of (metabolic) energy.

Address-message concept

Address-message concept refers to compounds in which part of the molecule is required for binding (address) and part for the biological action (message).

ADME

Abbreviation for **Absorption, Distribution, Metabolism, Excretion**. (See also **Pharmacokinetics**; **Drug disposition**).

Affinity

Affinity is the tendency of a molecule to associate with another. The **affinity** of a **drug** is its ability to bind to its biological target (**receptor**, **enzyme**, transport system, etc.) For pharmacological **receptors** it can be thought of as the frequency with which the **drug**, when brought into the proximity of a **receptor** by diffusion, will reside at a position of minimum free energy within the force field of that **receptor**.

For an **agonist** (or for an **antagonist**) the numerical representation of **affinity** is the reciprocal of the equilibrium dissociation constant of the ligand-**receptor** complex denoted K_A, calculated as the rate constant for offset (k_{-1}) divided by the rate constant for onset (k_1).

Agonist***

Catabolism consists of reactions involving endogenous organic substrates to provide chemically available energy (e.g., ATP) and/or to generate metabolic intermediates used in subsequent anabolic reactions.

Catabolite

A **catabolite** is a naturally occurring **metabolite**.

Clone*

A **clone** is a population of genetically identical cells produced from a common ancestor. Sometimes, "**clone**" is also used for a number of recombinant DNA (deoxyribonucleic acid) molecules all carrying the same inserted sequence.

Codon*

A **codon** is the sequence of three consecutive **nucleotides** that occurs in mRNA which directs the incorporation of a specific amino acid into a protein or represents the starting or termination signals of protein synthesis.

Coenzyme

A **coenzyme** is a dissociable, low-molecular weight, non-proteinaceous organic compound (often **nucleotide**) participating in enzymatic reactions as acceptor or donor of chemical groups or electrons.

Combinatorial synthesis

Combinatorial synthesis is a process to prepare large sets of organic compounds by combining sets of building blocks.

Combinatorial library

A **combinatorial library** is a set of compounds prepared by combinatorial synthesis.

CoMFA

See **Comparative Molecular Field Analysis**.

Comparative Molecular Field Analysis (CoMFA)**

Comparative molecular field analysis (CoMFA) is a **3D-QSAR** method that uses statistical correlation techniques for the analysis of the quantitative relationship between the biological activity of a set of compounds with a specified alignment, and their three-dimensional electronic and steric properties. Other properties such as hydrophobicity and hydrogen bonding can also be incorporated into the analysis. (See also **Three-dimensional Quantitative Structure-Activity Relationship** [**3D-QSAR**]).

Computational chemistry**

Computational chemistry is a discipline using mathematical methods for the calculation of molecular